The functional role of the conserved active site proline of triosephosphate isomerase

Marco G. Casteleijn§, Markus Alahuhta§, Katrin Groebel§, Ibrahim El-Sayed‡, Koen Augustijns‡, Annemie Lambeir‡, Peter Neubauer§, Rik Wierenga§

§ Oulu University; ‡ University of Antwerp

Triosephosphate isomerase (TIM) is a glycolytic enzyme with very high substrate specificity. TIM catalyses the interconversion of D-glyceraldehyde-3-phosphate (DGAP) and dihydroxyacteone phosphate (DHAP). The wild type enzyme is a dimer, and each subunit has the classical TIM-barrel fold.

Our studies are focusing on understanding the catalytic properties of the Wild Type (Wt-TIM) [1, 2] and applying this knowledge to our newly engineered enzymes.

Enzymology of P168A vs. wild type

We investigated the enzymological properties of wild type TIM and its P168A mutant regarding the loop-6 / loop-7 mechanism.

The results are summarized in figure 1, where the impact on conversions per second and affinity for 2PG can be seen.

Interestingly the affinity for the phosphate moiety of the substrate is not affected. The results clearly show the importance of proline 168 for catalytic activity.

This investigation, and of others [3, 4, 5], are of importance for protein engineering efforts on TIM, since only in the closed form the active site is competent for catalysis.
Structural information

Structural investigation of liganded and unliganded P168A mutant revealed two new observations. The conformational switch from “swung out” to “swung in” of the catalytic glutamate (Glu167) has not occurred upon ligand binding in the P168A mutant.

This clearly affects the mode of binding of 2PG where the mode of binding is “down”; unlike in wild type (Fig. 2).

The 2nd new observation is that the trigger for loop-6 closure is induced by the interactions of the PO₄ moiety of the ligand with residues in loop 7.

This switch of loop-6 is facilitated, or even triggered, by the movement of O (G211) in loop 7 as it clashes with CD (P168).

This occurs if the P168 side chain would not move on ligand binding (Fig. 2). This switch does not exist in the P168A mutant.

Conclusions

- Proline 168 is needed for conformational strain around the catalytic glutamate. This strain is transferred to the catalytic glutamate.
- Ligand binding triggers the conformational switch of the catalytic machinery needed for catalysis.

Abbreviations

DGAP (R)-glyceraldehyde-3-phosphate
DHAP Dihydroxyacetone phosphate
TIM Triosephosphate Isomerase

References

[1].Kursula et.al., 2003. JBC. 278, 9544 - 51

This project was financed by the Academy of Finland (project 53923)